Euphytica (2009) 165:597–606
DOI 10.1007/s10681-008-9791-5

Molecular mapping of *Cg1*, a gene for resistance to anthracnose (*Colletotrichum sublineolum*) in sorghum

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Received: 18 July 2008 / Accepted: 11 August 2008 / Published online: 26 August 2008

Abstract Anthracnose, caused by the fungus *Colletotrichum sublineolum* is one of the most destructive diseases of sorghum and has been reported in most areas where the crop is grown. Several control strategies have been developed but host plant resistance has been regarded as the most effective strategy for disease control. Here, we describe the search for molecular markers that co-segregate with *Cg1*, a dominant gene for resistance originally identified in cultivar SC748-5. To identify molecular markers linked with the *Cg1* locus, $F_{2:3}$ plants derived from a cross to susceptible cultivar BTx623 were analyzed with 98 AFLP primer combinations. BTx623 was chosen as the susceptible parent because it is also one on the parents used in creating RFLP and AFLP maps and BAC libraries for sorghum. Four AFLP markers that cosegregate with disease resistance were identified, of which *Xtxa6227* mapped within 1.8 cM of the anthracnose resistance locus and all four AFLP markers have been previously mapped to the end of sorghum linkage group LG-05. Sequence scanning of BAC clones spanning this chromosome led to the discovery that *Xtxp549*, a polymorphic simple sequence repeat (SSR) marker, mapped within 3.6 cM of the anthracnose resistance locus. To examine the efficacy of *Xtxa6227* and *Xtxp549* for marker-assisted selection, 13 breeding lines derived from crosses with sorghum line SC748-5 were genotyped. In 12 of the 13 lines the *Xtxa6227* and *Xtxp549* polymorphism associated with the *Cg1* locus was still present, suggesting that *Xtxp549* and *Xtxa6227* could be useful for marker-assisted selection and for pyramiding of *Cg1* with other genes conferring resistance to *C. sublineolum* in sorghum.

Keywords Anthracnose disease · Molecular markers · Genetic Map · BAC

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Introduction

Grain sorghum [*Sorghum bicolor* (L.) Moench] production can be limited by a number of biotic stresses. The foliar disease anthracnose, which is caused by the fungus *Colletotrichum sublineolum* (=*C. graminicola* P. Henn in Kabát and Bubk; Sutton 1980; Sheriff et al. 1995), is one of the most damaging diseases to infect sorghum (Ali and Warren 1992; Pastor-Corrales 1980). It was first reported in Texas in 1912 with significant outbreaks occurring sporadically in the U.S. since that time (Reyes et al. 1969; Warren 1986; Cardwell et al. 1987). Estimating grain yield losses due to anthracnose can often be difficult (Ngugi et al. 2000), but losses as high as 50% have been reported in susceptible cultivars (Harris et al. 1964; Powell et al. 1977; Thomas et al. 1995). Despite use of several methods for control, employment of host plant resistance is still considered the most effective disease control strategy (Warren 1986; Rosenow and Frederiksen 1982). Breeding, however, for stable host plant resistance has been difficult even in regions with endemic anthracnose because of the hypervariable nature of *C. sublineolum* along with strong environmental effects on symptom development and disease spread. Consequently, even though several sources of genetic resistance are known, an understanding of the basis for anthracnose resistance is still lacking.

Studies have examined anthracnose resistance in sorghum germplasm from the USDA-TAES Sorghum conversion program (Cardwell 1989). Coleman and Stokes (1954) reported that resistance to anthracnose in sorghum line Sart is encoded by two closely linked dominant genes, each conferring resistance to different phases of the disease. Jones (1979) and Tenkouano (1993) both reported that resistance to anthracnose in SC326-6 was controlled by a single genetic locus with multiple allelic forms, while Boora et al. (1998) reported that a single recessive gene conferred resistance in SC326-6. Mehta et al. (2005) identified four converted lines that displayed unique, but simply inherited sources of anthracnose resistance. Some of these sources showed dominant resistance while others were recessive. Of these germplasm sources, the resistance from SC748-5 was the most stable across environments. Segregation studies by Mehta et al. (2005) using 235 lines in 1999 and 146 lines in 2000 fit a 3:1 ratio of resistant to susceptible phenotypes in the F_2 generation suggesting that a single dominant gene, *Cg1*, in sorghum line SC748-5 confers resistance to anthracnose.

Identification of markers linked to anthracnose resistance gene *Cg1* would facilitate marker-assisted selection of resistance in breeding populations. Conventional selection for anthracnose resistance in sorghum has been hampered by difficulties in obtaining the proper disease pressure to permit accurate identification of resistant plants (Mehta 2002). Molecular markers linked to gene(s) of interest are one possible strategy to permit selection for anthracnose resistance without concern for pathogen pressure. A vast array of genome resources for sorghum have been developed in the past 10–15 years including several high-density genome maps (Klein et al. 2000; Bowers et al. 2003) and these resources have facilitated the mapping of loci controlling disease incidence. DNA-based molecular markers delimiting disease resistance loci in sorghum have been reported for head smut (Oh et al. 1994), downy mildew (Gowda et al. 1995; Agrama et al. 2002), leaf blight (Boora et al. 1999), and grain mold (Klein et al. 2001). Boora et al. (1998) also identified markers linked to a recessive gene conditioning anthracnose resistance, but the RAPD markers identified have not been placed on a map. Recently, Singh et al. (2006) were able to show that an anthracnose resistance gene in sorghum line G73 maps the long arm of chromosome 8 on the basis of linked RAPD and SCAR markers.

The objective of this study was to identify molecular markers tightly linked with the locus for anthracnose resistance in sorghum line SC748-5. This source of anthracnose resistance would have utility for the sorghum hybrid seed industry since the gene has been shown to confer stable resistance across multiple environments (Mehta 2002; Mehta et al. 2005). The mapping results are discussed in the context of using linked markers to introgress *Cg1*-based resistance into elite breeding material.

Materials and methods

Germplasm development

The materials used in this study were selected from an $F_{2:3}$ population developed from the cross between converted line SC748-5 (resistant parent, *Cg1*/*Cg1*) and elite inbred BTx623 (susceptible parent, *cg1*/*cg1*).

The F_1 cross was made in College Station, Texas. An F_2 population was created by selfing F_1 hybrid plants in a winter nursery in Guayanilla, Puerto Rico. Individual F_2 plants were self-pollinated to produce $F_{2:3}$ families, which were grown and scored for resistance. These families were initially evaluated through artificial inoculation for anthracnose response for 5 years (1996, 1997, 1999–2001) at the TAMU Research Farm, College Station, TX. Environmental factors unfavourable to disease development during these years resulted in high rates of escape from disease in susceptible families. Therefore, lines were re-screened in 2003 at College Station and Cairo, GA, where the infection was epidemic due to conducive environmental conditions (warm and humid) for anthracnose disease expression.

Inoculation of *colletotrichum sublineolum* and disease investigation

The characterization of the segregating population for anthracnose was based on response to *C. sublineolum* isolate 430BB-85. Isolate 430BB-85 was chosen because of its aggressive virulence and common presence in Texas (Cardwell 1989). This isolate was cultured on oatmeal agar (7.25%, w/v) fortified with ampicillin (0.1%, v/v) and streptomycin (0.1%, v/v) at 23.8°C. The culture was grown under constant fluorescent light (60 μE m^{-2} s^{-1}) to limit mycelial growth and induce sporulation (Tenkouano 1993). Two week-old plates were flooded with distilled water, and conidia were gently scraped off the plates. Spore density was determined with a haemocytometer, and adjustments were made to reach the desired inoculum concentration of 10^6 conidia ml^{-1}. The wetting agent Tween 20 was added to the inoculum (0.5 ml l^{-1}) to reduce surface tension. In each experimental year, inoculation was achieved by spraying approximately 3–5 ml of the conidial suspension (10^6 conidia ml^{-1}) onto the leaves and the whorl of each plant.

Field inoculations were made during the transition from growth stage 1 to growth stage 2 (Pauli et al. 1964), when approximately seven to nine leaves were fully developed. In all environments examined, effective inoculation and subsequent infection were obtained on all susceptible check plots with disease symptoms appearing within 2 weeks following the inoculation. In 2003, inoculations were made by dropping *C. sublineolum*-colonized sorghum grains into the whorl of each plant (Erpelding and Prom 2006). The plots in College Station were irrigated as needed until grain fill was completed.

Anthracnose ratings in the field were made both before and after flowering. Disease assessments were conducted 30 days post-inoculation and thereafter on a weekly basis for four consecutive weeks until flowering. Ratings were based on a scale of 1-to-5 modified from Pande et al. (1991), where 1 = no symptoms or chlorotic flecks on leaves; 2 = hypersensitive reaction (reddening or red spots) on inoculated leaves but no acervuli formation and no spreading to other leaves; 3 = lesions with small acervuli in the center of leaves up to one third of plant length from the bottom; 4 = necrotic lesions with acervuli on all leaves except the flag leaf; and 5 = necrotic lesions with abundant acervuli on the whole plant. In the $F_{2:3}$ plots, a family was scored as susceptible if all plants within the plot were rated susceptible; a family was considered to be segregating if one or more, but not all, plants within the plot were susceptible, and a family was considered resistant if all plants within the plot were completely free of sporulating lesions induced by the pathogen.

DNA extraction and AFLP analysis

Seeds of 10–20 individuals per family were grown in the greenhouse and leaf tissues from about two week-old seedlings were harvested for genomic DNA extraction [FastDNA kit protocol (Q.BIOgene, BIO 101, Carlsbad, CA)]. DNA from 71 $F_{2:3}$ families (29 resistant, 29 susceptible and 13 heterozygous) was used for marker analysis. For AFLP analysis, DNA samples were digested with the restriction endonuclease pairs *Eco*RI and *Mse*I or digested sequentially with *Pst*I and *Mse*I. AFLP template preparation and PCR reaction conditions were as described (Klein et al. 2000). Ninety-eight AFLP primer combinations, each with 3 base extensions (+3/+3) were initially examined in 12 resistant and 12 susceptible $F_{2:3}$s along with the inbred parents BTx623 and SC748-5. Line IS3620C was also included so that both parents of a sorghum genomic map (BTx623 and IS3620C) could be compared for polymorphism and co-segregation in the susceptible/resistant $F_{2:3}$s (Menz et al. 2002). The products were examined using a dual-dye LI-COR 4200 IR^2 gel detection system (LI-COR Inc., Lincoln, NE). Primer information and PCR

conditions for all markers in this study were reported by Menz et al. (2002) and can be viewed at the TAMU-USDA Sorghum Genome website (http://sorgblast3.tamu.edu/).

Co-segregation analysis

From an overall population of 146 F_2 families scored, 29 that gave only susceptible progeny, another 29 that gave only resistant progeny and 13 that were segregating for disease response were used to identify cosegregating AFLP and SSR markers. Mapping data were obtained by visual scoring of gels based on amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs). Linkage between the markers and *Cg1* was analyzed using MAPMAKER/EXP version 3.0b. Map distances in centimorgans (cM) were calculated from recombination frequencies using Kosambi's mapping function (Kosambi 1944) in MapMaker/Exp. LG nomenclature is according to Kim et al. (2005).

BAC analysis and marker discovery

To determine the physical position of *Cg1*, linked AFLP markers were cross referenced to an integrated genetic/physical map of sorghum (http://sorgblast3.tamu.edu/), and four AFLP markers placed *Cg1* on LG-05. To identify linked SSRs, 10 BAC clones spanning this region of LG-05 were sequence scanned as previously described (Klein et al. 2003), and SSRs were identified using SSRIT (http://www.gramene.org/db/searches/ssrtool). SSR primers were designed from the sequences using PRIMER 3 (Center for Genome Research, Whitehead Institute, Mass., USA). SSR primer pairs derived from each of the 10 BAC clones were initially screened for the ability to detect polymorphisms in the parental lines, 12 resistant and 12 susceptible individuals by electrophoresis of the amplified products in 4% super fine resolution (SFR) agarose (MidWest Scientific) gels. PCR conditions were as described in Menz et al. (2002), with annealing temperatures ranging from 2°C above to 2°C below the T_m of the primers. Only one of the 10 primer pairs, SSR *Xtxp549*, amplified polymorphic products in the parental lines. The PCR products of SSR *Xtxp549* showed apparent cosegregation with the 12 resistant and 12 susceptible individuals so were tested in all 29 resistant 29 susceptible and 13 heterozygous $F_{2:3}$s using a dual-dye LI-COR 4200 IR^2 gel detection system (LI-COR Inc., Lincoln, NE) by labelling the forward primer with one of the two IR fluorescent dyes.

Results

Disease development and rating

Colletotrichum sublineolum is capable of infecting all above ground tissues of the sorghum plant; however, the foliar disease is most widely distributed. Foliar infection can occur at any stage of plant development, but symptoms are generally observed 40 days after seedling emergence. Characteristic symptoms on susceptible cultivars include small circular to elliptical spots or elongated lesions and as the fungus sporulates, fruiting bodies (acervuli) appear as black spots in the center of the lesions (Thakur and Mathur 2000). In the present study, for disease rating the symptom types were qualitatively categorized as either resistant (ratings of 1 or 2) or susceptible (ratings of 3–5). The difference between a resistant and susceptible response is the presence of acervuli on the leaves, which indicates successful reproduction of the pathogen. Scoring multiple times enhanced the probability of detecting sporulating lesions, especially in those $F_{2:3}$ rows where most plants were resistant.

AFLP and BAC analysis

To identify AFLP markers showing co-segregation with the *Cg1* locus, genomic DNA samples from $F_{2:3}$ families were analyzed. Ninety-eight AFLP (*Xtxa* prefix) primer combinations (+3/+3 selection) revealed four markers that could be visually scored as segregating with anthracnose resistance; *Xtxa6227* (P39M56-94.4[1]), *Xtxa3137* (E42M48-260.8), *Xtxa2303* (E63M60-220.4), and *Xtxa3588* (E61M61-206.2) (Standard Code for AFLP Primer Nomenclature registered trademark of Keygene N.V.). The segregation through a subset of $F_{2:3}$ families of a representative marker (*Xtxa6227*) is shown in Fig. 1 (Plate 1 & 2), and a LOD 3.00 regional linkage map is shown in Fig. 2. Linkage analysis of 71 $F_{2:3}$ families indicated that *Xtxa6227* was 1.8 cM from the *Cg1* locus. All four AFLP markers displaying linkage to *cg*1 have been previously mapped in an $F_{6–8}$ recombinant

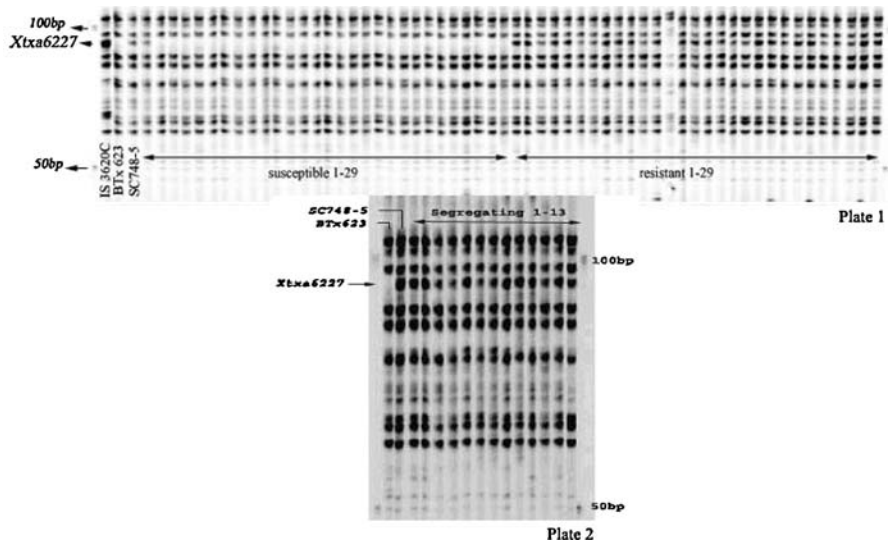

Fig. 1 Plate 1 & 2 Co-segregation of AFLP marker *Xtxa6227* and the *Cg1* allele in $F_{2:3}$ progeny derived from the cross of BTx623 and SC748-5. AFLP templates from parental inbreds BTx623 (*cg1cg1*) and SC748-5 (*Cg1Cg1*) and IS3620C (mapping parent) were run as controls to aid in the identification of polymorphic bands. The *faint lane* represents a failed PCR reaction. The *arrow* to the left and right indicates the position of AFLP marker *Xtxa6227*. Fluorescent-labeled molecular-weight markers (LI-COR) were run on both ends and their sizes (bp) are shown at the margin of the gel

inbred line population of sorghum, where BTx623 was also a parent (Menz et al. 2002). Although each of the AFLPs is associated with LG-05, all were 'placed' distal to the last framework marker at one end of the linkage group and hence, their precise map positions have not been determined. In addition, no SSR loci previously mapped to this region of LG-05 were polymorphic in the parents of the anthracnose mapping population.

To identify additional SSRs that map to the distal arm of LG-05, 10 BAC clones spanning this region were sequence scanned and simple sequence repeats identified. The most informative SSR identified was *Xtxp549*, which represents a $(CT)_8$ repeat motif in BAC clone 117c5. The segregation pattern for *Xtxp549* showed close co-segregation (3.6 cM) with anthracnose resistance (Figs. 2 and 3-Plate 1 & 2). Dominant AFLP marker *Xtxa6227* is linked in coupling phase with *Cg1* at 1.8 cM, while the other dominant markers, *Xtxa3137*, *Xtxa2303*, and *Xtxa3588*, are loosely linked in repulsion phase with the resistance allele at 10, 19 and 32 cM, respectively, on the opposite side of *Cg1*.

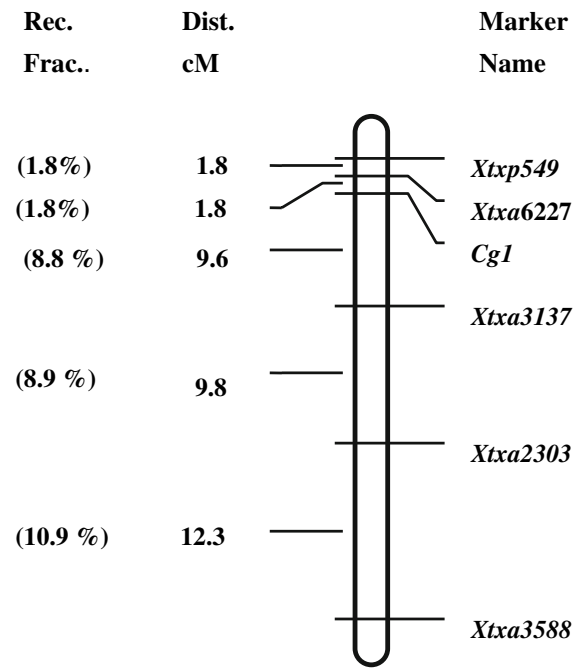

Fig. 2 Regional linkage map displaying the AFLP (*Xtxa*) and SSR (*Xtxp*) markers linked to resistance gene *Cg1* near one end of sorghum LG-05

Marker implementation

To determine the potential applicability of *Xtxa6227* and *Xtxp549* for marker-assisted transfer of *Cg1* to elite germplasm, 13 unique advanced breeding lines derived from sorghum line SC748-5 were genotyped with *Xtxa6227* and *Xtxp549* (see Table 1). Each of

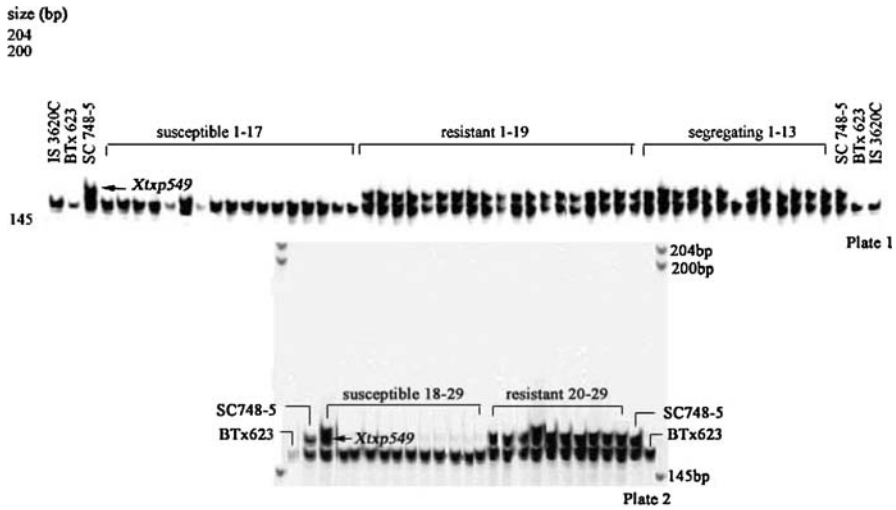

Fig. 3 Plate 1 & 2 Co-segregation of SSR *Xtxp549* and resistance gene *Cg1* in $F_{2:3}$ progeny derived from the cross of ATx623 and SC748-5. Genomic DNA from parental inbreds BTx623 (*cg1cg1*) and SC748-5 (*Cg1Cg1*) were included to aid in the identification of parental alleles for *Xtxp549*. The molecular weight of the *Xtxp549* allele was 152 bp (BTx623) or 155 bp (SC748-5) Fluorescent-labeled molecular-weight markers (LI-COR) are shown at the margins of the gel

these lines represents an F_{5-6} line that originated from a cross of SC748-5 with an elite inbred. Anthracnose resistance was visually scored during each selfing generation and resistant family members were advanced to the next generation. Each of these 13 advanced breeding lines was screened with *Xtxa6227* and *Xtxp549* as a preliminary determination of the utility of AFLP and SSR for marker-assisted introgression of *Cg1*. In 12 of the 13 F_{5-6} inbred lines, *Xtxa6227* and *Xtxp549* were still associated with *Cg1*, so correctly predicted the phenotype for anthracnose disease resistance. In the seventh line, which was also resistant indicating the presence of *Cg1*, the AFLP allele of *Xtxa6227* and SSR allele of *Xtxp549* found in SC748-5 is not present (Fig. 4-Plate 1 & 2).

Discussion

A dominant gene from segregating F_2 progeny of the cross BTx623*SC748-5 has been shown to confer resistance to the foliar disease anthracnose in multiple locations over several years (Mehta et al. 2005). Stability of the single gene segregation pattern across several environments made it possible to produce a useful population for demonstrating the mode of inheritance. The ability to identify genetic markers very closely linked to another locus is critically dependent on the ability to perfectly score the segregation of this trait within a mapping population. In the present situation, accurate disease phenotypes were not obtained initially due to environmental factors unfavourable to disease development. In a detailed examination of disease development for sorghum anthracnose, Wharton et al. (2001) observed that early infection processes including spore germination, differentiation of appresoria, and penetration of epidermal cells of sorghum leaf sheaths were the same in compatible and incompatible interactions. Host responses in both resistant and susceptible cultivars lead to the accumulation of pigments around the sites of infection. These conditions tend to cause mis-scoring of F_3 plants, with escapes being assigned a resistant rating. Based solely on anthracnose field scores obtained in this manner (College Station, 1996, 1997, 1999–2001), AFLP marker screening of the mapping population yielded inconsistent linkage results and failed to identify any closely linked molecular markers (data not shown). Mehta (2002) reported that the degree of infection on susceptible plants within susceptible or segregating $F_{2:3}$ lines was very low compared to the infection observed on susceptible checks. This may indicate a lower level of viable inoculum due to the presence of resistant lines throughout the field plots or segregation of other genes that modify disease symptoms or severity; nevertheless, this fact also made accurate scoring difficult. To alleviate

Table 1 Anthracnose disease phenotype and marker genotype on sorghum advanced breeding lines with SC748-5 background

Source	Pedigree	Generation	Disease phenotype	*Xtxa6227* & *Xtxp549*
05CA1428-1	(RTx2918*SC748-5)-BE4-CS1	F5	R	M^R
05CA1521-1	(Tx436*SC748-5)-BE5-CS1	F5	R	M^R
05CA2991-1	(SC176-14E*SC748-5)-F1-CSF2-CS1	F4	R	M^R
05CA2997-1	(SC176-14E*SC748-5)-F1-CSF2-CS7	F4	R	M^R
05CS734-1	(SC748-5*90B2662)-BE10-CA1-CA1	F6	R	M^R
05CS737-1	(SC748-5*90B2662)-CS7-CA2-CA1	F6	R	M^R
05CS746-1	(Tx2891*SC748-5)-BE3-CA1-CS1	F6	R	M^S
05CS754-1	(Tx430*SC748-5)-CS6-CA2-CA1	F6	R	M^R
05CS6932-1	(RTx2918*SC748-5)-CS5-CA1	F5	R	M^R
05CS6980-1	(RTx2918*SC748-5)-CS5-CA1	F5	R	M^R
05CS7028-1	(RTx2918*SC748-5)-CS5-CA1	F5	R	M^R
05CS7033-1	(SC748-5*RTx2919)-CS6-CA2	F5	R	M^R
05CS7066-1	(SC748-5*RTx2919)-CS6-CA2	F5	R	M^R

R = resistant

M^R = showing the marker allele from the anthracnose resistant parent

M^S = showing the marker allele from the anthracnose susceptible parent

Xtxa6227—AFLP marker

Xtxp549—SSR marker

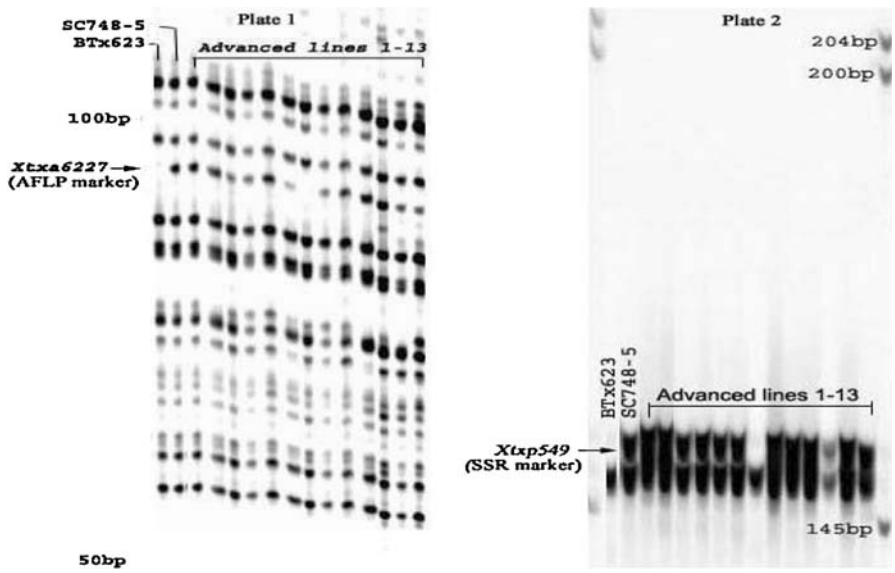

Fig. 4 Genotyping of anthracnose-resistant advanced breeding lines with AFLP *Xtxa6227* (Plate 1) and SSR *Xtxp549* (Plate 2). Breeding lines represent genetic material derived from crosses with SC748-5, which were selected for resistance to anthracnose during each successive backcrossing (see Table 1). Genomic DNA from parental inbreds BTx623 (*cg1cg1*) and SC748-5 (*Cg1Cg1*) were included in the analysis to aid in the identification of parental alleles. Fluorescent-labeled molecular-weight markers (LI-COR) are shown at the margins of the gel

this problem, anthracnose disease ratings were rescored again under more optimal field conditions in 2003, under irrigation at College Station and in Cairo, Georgia where environmental conditions (warm and humid) conducive for disease development prevailed. In addition, an alternate inoculation system that has

proven to be extremely effective was used, (Erpelding and Prom 2006) and greatly enhancing the ability to differentiate resistant, segregating and susceptible families. When combined with data from earlier years sufficient numbers of homozygous resistant and susceptible F_2 progeny rows were identified to permit mapping with confidence. This reclassification of the mapping population clearly revealed the mis-classification in earlier disease phenotyping, and also permitted the identification of AFLP and SSR markers that co-segregate with the *Cg1* resistance gene.

The use of DNA markers is an effective way of obtaining essential information on the genomic region around a given gene (Messeguer et al. 1991) and ultimately isolating the gene of interest (Van Dommelen et al. 2002). Previously, Boora et al. (1998) identified RAPD markers linked to a recessive form of anthracnose resistance in sorghum converted line SC326-6. While molecular tags would be especially useful for tracking a recessive allele during introgression into parental lines for creating hybrids, the RAPD polymorphisms were not seen in other crosses, including the cross used for mapping. Hence, the map location of this locus remains unknown, preventing the potential identification of other useful markers from the same region. The appeal of the source of anthracnose resistance identified in SC748-5 relates primarily to its stability of resistance when tested across multiple locations. In addition to the stability of resistance displayed in College Station (TX) and Cairo (GA), *Cg1* conferred stable resistance at other locations including Rower and Pine Tree (AR), Mansa, Zambia and Bamako, Mali (Mehta 2002). This type of resistance is well suited for introgression into existing inbred lines to confer resistance in hybrids. The present identification of the distal region of sorghum LG-05 as the genomic location of *Cg1* will facilitate the introgression of resistance into elite inbreds. The fact that another anthracnose resistance gene has been located to LG-08 (Singh et al. 2006) further illustrates the usefulness of DNA tags to identify alternate sources of resistance and the potential for gene stacking.

The results reported here detail several AFLPs and a SSR that are linked to the *Cg1* locus. The closest marker to the *Cg1* locus was the AFLP marker *Xtxa6227* mapping at a distance of 1.8 cM. This dominant marker was linked in coupling phase with the resistant allele *Cg1* which is critical for the use of a dominant marker for trait introgression. While it would be possible to use *Xtxa6227* as a marker, its use requires generation of AFLP templates, followed by primer ligation and two rounds of PCR amplification. Sequencing of the AFLP product could serve as a starting point for developing a direct PCR marker, but identifying useful polymorphisms could entail considerable work. By contrast, SSR marker *Xtxp549* represents the first in a series of polymorphic linked markers that can be directly used to track introgression of *Cg1* into elite inbreds, even in the absence of the pathogen. This SSR resides 3.6 cM distal to the *Cg1* locus and even though *Xtxp549* maps further from the *Cg1* locus than does *Xtxa6227*, the simplicity of using a SSR will offset the greater chance of error for use in initial screening. In addition, since any given marker may not be polymorphic between SC748-5 and an elite inbred, a series of markers (SSRs, Indels, SNPs) that flank the *Cg1* locus would ultimately be necessary for efficient introgression of disease resistance into elite germplasm. The sequencing of the sorghum genome that commenced in 2006 (Kresovich et al. 2005; http://www.jgi.doe.gov/sequencing/why/CSP2006/sorghum.html) will greatly facilitate the discovery of potential markers residing in this and other trait loci of interest. With the emerging sequence of the sorghum genome, the mapping and subsequent transfer of selected traits will become a less daunting task, and the vast gene pool residing in the sorghum germplasm collection will be more efficiently utilized in breeding programs.

Preliminary examination of the utility of *Xtxa6227* and *Xtxp549* in a marker-assisted breeding scheme appears promising. Twelve of thirteen advanced breeding lines that were bred for anthracnose resistance were correctly genotyped as encoding *Cg1*. These results also provide supportive evidence for the distal region of sorghum LG-05 as encoding *Cg1*, and further indicate that the region tagged by *Xtxa6227* and *Xtxp549* was introgressed into the majority of these advanced breeding lines. The line that failed to be classified as resistant by *Xtxp549* may represent a crossover event between *Cg1* and *Xtxa6227* and *Xtxp549*. Alternatively, it is possible that a second gene or gene combination provides resistance in this line. Although numerous genomic regions of SC748-5 likely still reside in these advanced breeding lines, the region flanking *Xtxa6227* and *Xtxp549* survived many selfing generations which is encouraging for future marker-assisted breeding schemes.

Resistance imparted by a single gene is often overcome by the pathogen as a result of mutation or pre-existing genetic diversity and sexual recombination (McDonald and Linde 2002). Hence, cultivar diversification, cultivar mixtures, multi-lines, and deployment of different resistance genes have been used in attempts to increase the durability of resistance. Marker-assisted selection can help in pyramiding different anthracnose resistance genes which should allow individual loci to have increased durability. Since several heritable sources of resistance have been identified for anthracnose (Wiltse 1998; Mehta 2002), efforts are presently under way to identify other genes for resistance to anthracnose using SC155-14E and BTx378 as potential sources. Finally, the present work and future related investigations will extend the sorghum functional map for pathogen resistance.

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